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A NEW CRYPTIC SPECIES OF *ODONTOLOXOZUS* (NERIIDAE:DIPTERA) FROM THE CAPE REGION OF BAJA CALIFORNIA SUR (MEXICO)

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Abstract.—A new species of *Odontoloxozus* was discovered from the cape region of Baja California Sur. Differences between *O.* ***pachycericola*** n. sp. and *O. longicornis* Coquillett are described for adult and larval morphology, cytology and mating behavior. The two species hybridize readily in the laboratory and produce sterile hybrids. Differences in chromosome number, translocations, inversions and differences in degree of ectopic pairing of salivary chromosomes distinguish the species cytologically. Adults may be distinguished by differences in numbers of anepisternal spots, larvae differ in numbers of papillae on anterior spiracles. In analysis of copulation times for intra- and interspecific crosses, hybrids demonstrated clear reproductive differences in the species.

Closely related and morphologically similar species have been called cryptic species. Studies of these species have frequently produced interesting and useful information concerning population genetics and behavior as they interact in the processes of evolution. Reproductive isolation and the resulting integrity of gene pools may result from numerous combinations of genetic and ecological factors (Bush, 1975). While sibling or cryptic species may present serious problems to taxonomists, the value of these taxa to systematic zoology has been shown to be extensive (e.g. Dobzhansky and Epling, 1944; White, 1973).

In this study we describe *Odontoloxozus pachycericola* n. sp. based on evidence from adult and larval morphology, hybrid sterility, cytogenetics, and mating behavior. This last evidence was actually the first discovered during a study, following Mangan (1979), of the mating behavior of *O. longicornis* Coquillett in which males of *O. longicornis* were found to have difficulty mating with females from populations collected in the cape region of Baja California Sur, Mexico. We feel that evolution in this family is particularly interesting in that there are ecological and genetic parallels between these neriid species and better known species of the *nannoptera* and *repleta* species groups of *Drosophila* (Heed, 1982; Mangan, 1982) which co-inhabit cactus substrates with *Odontoloxozus* spp. (Mangan, 1984).

MATERIALS AND METHODS

Odontoloxozus longicornis adults used in this study were collected from three locations. Collections were made in the Tucson vicinity of Pima County, Arizona

by placing vials over males and females on saguaro cacti (*Carnegea gigantea* (Engelm.)) and by rearing larvae from infested necrotic limbs of the cactus. Adults from a coastal region north of Bahia Kino, Sonora were reared from necrotic sections of agria cactus (*Machaerocereus gummosus* (Engelm.)) and cardon cactus (*Pachycereus pringlei* (S. Wats.)). Mazatlan populations from Sinaloa were reared from necrotic sections of hecho cactus (*Pachycereus pectin-aborigium* (Engelm.)). These samples were all collected in mid-March of 1977 and 1978. Adults of *O. pachycericola* were reared from senita cactus (*Lophocereus schottii* (Engelm.)) and cardon cactus collected in March of the same years, further specimens were taken as adults from cactus stems and pinned during the 1978 collections.

Flies were maintained on *Drosophila* medium (banana, malt extract, yeast extract, agar) for several generations in population cages or in half pint milk bottles. This medium had to be periodically replenished (2 or 3 times in the 3–5 week development period). Dry tissue paper was inserted into the bottles to provide suitable pupation sites.

Third stage larvae and mature adults were examined for morphological characters to distinguish the populations. Larvae were softened for 20–24 hours in 30% KOH for removal of cephalopharyngeal skeleton and spiracles. Male genitalia were boiled in 30% KOH for 3–5 minutes before dissection. Differences between the species, and between the species and hybrids, were tested using appropriate "*t*" tests for comparisons of mean values after transformation in accordance with standard statistical procedures.

Third stage larvae were used for analysis of polytene chromosomes. Salivary glands were removed, fixed in sodium citrate (1%) then stained and squashed in acetic orcein. Karyotypes were determined from brain cells removed at the same time. These were also fixed in sodium citrate, then stained for 3 to 4 hours in acetic orcein and squashed. Testes were removed from male pupae or newly emerged adults in insect saline, teased apart, and stained in acetic orcein for 45 minutes.

Pair mating tests for production of hybrids and analysis of behavior were performed by placing 3 to 5 day old virgin males and females in 8 dram vials with *Drosophila* medium provided for adult nutrition. Mating times were recorded for at least 6 pairs when possible for each of the combinations of reciprocal crosses among the 4 collection locations. Due to lack of sufficient numbers of virgin flies of the same age, certain crosses could not be made. Slow development in the Tucson populations resulted in a shortage of females, while the Baja California Sur (La Paz) samples produced few males at the time the other groups were emerging. It was decided to test within site matings completely, then look at between site matings with emphasis on crosses between sites in which pairs showed extended mating times or populations had different karyotypes. Mating times were recorded using a stopwatch with time measured as period of genital contact. First and repeated matings were recorded for each pair for a period of 4 hours of observation.

Results

Analysis of data and observations for comparisons of genetics and behavior of these four populations is divided into three sections. Morphological comparisons are given first, as these provide the basis for description of *O. pachycericola*. Adult

and larval stages of *O. longicornis* were described by Olsen and Ryckmann (1963); additional description of third stage larval characters was provided in Steyskal (1965). The description of *O. pachycericola* follows, then a description and comparison of the karyotypes and polytene configurations of the two species. The last section of results includes analysis of mating behavior for inter- and intra-population crosses.

Morphological comparisons and diagnoses.—Analysis of thoracic pigmentation (spot number) and third instar papilla number for both anterior spiracles (Table 1) allows separation of adults and third stage larvae of the species. Spots on the left anepisternum were used as this surface is small enough to provide a reasonable number of spots to count and is normally visible on mounted specimens. These data show that *O. longicornis* average about twice as many spots as *O. pachycericola.* Differences between species are highly significant (i.e. there is no overlap), and there is no significant variation among the 3 *O. longicornis* populations ($P > 0.5$). Spot number does not differ significantly between hybrids and *O. pachycericola* ($t = 1.73$, $P > 0.05$), indicating possible dominance for low spot number. Since hybrids of both sexes were sterile, no further investigations of this trait were performed.

Number of papillae on the anterior spiracles is also diagnostic for the two species, though the difference is not as great. The hybrid was significantly different from *O. longicornis* for all *O. longicornis* populations pooled. However it was not different from the Tucson population when compared alone. Dominance for this trait appears to be incomplete in the hybrids.

Odontoloxozus pachycericola Mangan and Baldwin, New Species

Description.—*Head:* Brown, heavy grey pruinosity anterior to compound eye, remaining surface lightly dusted or bare. Genae lightly dusted, post-orbital surface heavily pruinose. Base of antennae shiny brown-black. Antennae porrect, brown, lightly pruinose.

Thorax: Scutum grey, pruinose with three median, longitudinal dark brown vittae. Center vitta extended to presutural area of scutellum, lateral vittae end just posterior to transverse suture. One row of dorsocentral bristles lateral to vittae, occasional bristles scattered longitudinally over median third of scutum. Lateral surfaces of scutum appearing slightly darker than medial dorsal surface due to a patch on each dorso-lateral surface and numerous dorso-central, supra-alar, and intra-alar bristles, each with a dark patch at base. Pleura and coxae grey, densely pruinose. Anepisternum with 17 to 25 spots (Table 1). Legs with trochanters, femora and tibiae yellow-brown to brown. Femora distally with two dark bands; tibia slightly darker at distal end; tarsi dark brown with black bristles. Wings identical to *O. longicornis.*

Abdomen: Terga laterally dark grey on median and posterior surfaces, pruinose on anterior surfaces. Male and female genitalia yellow, male surstylus with 4 gripping lobes at end of elongated epandrium. Aedeagus coiled inside epandrium. Female with elongate, retractable ovipositor tapering from bulbous base to narrow distal portion. Genitalia of both sexes not distinguishable from *O. longicornis.*

Holotype ♂ and 5 paratypes, 3 ♀ and 2 ♂, from El Centenario, Baja California, Mexico, 15 km west La Paz Baja California. Reared ex. *Lophocereus schottii*

Table 1. Morphological comparisons of *O. longicornis* and *O. pachycericola* and their hybrids.

Species	Character	Mean	SD	CV (%)	N
O. pachy.	anepistern. spots	20.82	3.12	14.9	28
O. long. (Maz.)	anepistern. spots	43.80	6.13	13.9	20
O. long. (Kino)	anepistern. spots	46.59	7.28	15.6	23
Hybrids	anepistern. spots	23.42	4.31	15.6	7
O. pachy.	spir. papillae	27.60	1.90	6.9	30
O. long. (Maz.)	spir. papillae	35.53	2.08	5.8	30
O. long. (Kino)	spir. papillae	32.81	2.45	7.4	16
Hybrids	spir. papillae	31.69	3.19	10.0	16

(Engelm.) April 1975. Deposited in National Museum of Natural History, Washington, D.C.

Etymology: The specific epithet *pachycericola* is derived from the subtribe Pachycerinae of the Cactaceae (see Gibson and Horak, 1978), the "thick stem" columnar cactus taxon in which the species commonly breeds.

Odontoloxozus longicornis and *O. pachycericola* are easily separated by differing numbers of bristles (with dark patches at their base) on the lateral thoracic areas of adults. Third stage larvae and puparia are separated by differing numbers of papillae on the anterior spiracles. Because both of these characters are readily quantifiable, they are used for identification of species and may be useful for detection of hybridization in natural communities. Neither of these characters changed in response to any of the environmental conditions including changes from cactus diet to artificial medium or to changes in temperature and humidity.

Chromosomal analysis.—Idiograms for the two species are given in Fig. 1 along with relevant percent of total chromosome length and centromeric indices for autosomes and X chromosomes. The species can easily be identified by differences in chromosome number and morphology. The karyotype of *O. longicornis* consists of 5 submetacentric autosome pairs; sex chromosomes are more heavily stained and the X chromosome is polymorphic. The Y chromosome has only been observed in testes material. It is about 50% the size of autosome 5 and darkly stained. Females in the Kino population are frequently heterokaryotypic. The karyotype of *O. pachycericola* has 6 autosome pairs, all are more submetacentric than in *O. longicornis* and 2 autosomes (V and VI) are smaller than any *O. longicornis* autosomes. We did not find any karyotypic polymorphism in 30 individuals of this species.

Polytene chromosomes of the two species and hybrids are shown in Figs. 2–4. Chromosomes of *O. longicornis* (Fig. 2) generally show extensive ectopic pairing and sometimes have a distinct chromocenter. Chromosome ends of this species are usually attached, making identification difficult. Polytenes of *O. pachycericola* (Fig. 3) normally do not show ectopic pairing, ends are free and arms can be easily recognized. For the hybrids (Fig. 4) chromosomes pair along regions of homology, but there is no synapsis. In hybrids, 1 chromosome (A) shows homeologous pairing for most or all of the length. In others (B) there is pairing but loops are present indicating deletions, translocations or other differences in the pair. Other chromosomes (C) show a complex of homologies suggesting a number of rearrange-

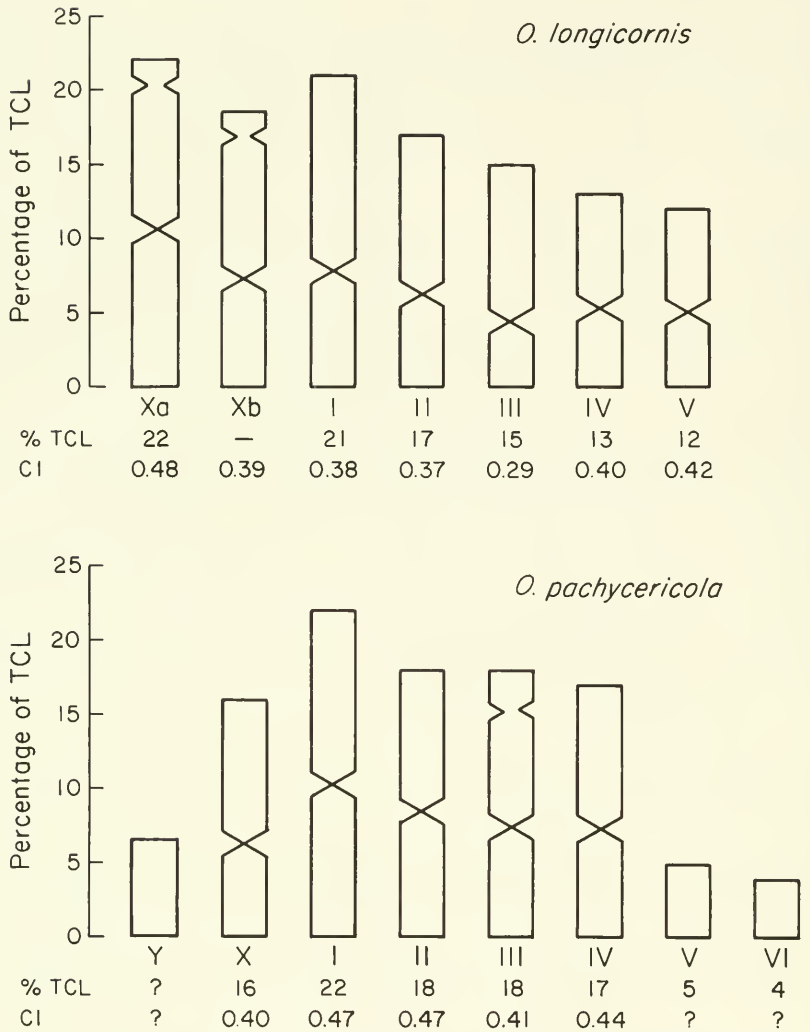

Fig. 1. Idiograms of the two *Odontoloxozus* species with relevant chromosome data. The Y chromosome in *O. longicornis* is similar in morphology to *O. pachycericola* but has not been measured in a complete karyotype.

ments and Robertsonian changes. Hybrids formed from crosses among the 3 populations of *O. longicornis* did not show any polymorphism in polytenes.

Mating time analysis.—Mating behaviors of *O. longicornis* and *O. pachycericola* were observed under field and laboratory conditions. Males mount females by jumping to a position above the female or by pulling the female under them with their forelegs, neither display or dancing behavior has been noted. Males use their hind legs to position the terminal abdominal segment of the female while standing over the female. Females may escape by running from the guarded position without copulating; males rarely follow to make further attempts (Mangan, 1979).

Variation in mating time, measured as the total period of genital contact, is summarized in Fig. 5 and analyzed in Table 2 for populations of *O. longicornis*

Fig. 2. Polytene chromosomes from salivary glands of *O. longicornis*.

in the Sonoran desert and *O. pachycericola* from the cape region of Baja California. These data show apparent differences in interpopulation crosses which affect mating times. Dissections of three females from matings interrupted after thirty seconds showed that sperm is transferred in this short time. Length of the copulatory period is apparently determined by the male. No differences in male genital morphology, which could cause difficulties in insertion or extraction of the aedeagus, were noted. All matings were terminated by the males extracting the aedeagus with little apparent effort.

Analysis of mating time data was performed using a series of simple analysis of variance models for each component (sex and population) and type of mating (intra- or inter-specific). There were a total of 133 tests. For each type of fly, classified by locality of source population and sex, all matings either involved that type or did not. The analysis model treated mating time as a dependent variable with fly type (sex and locality) as the independent variable. Since there were different numbers of matings for each type, the powers of these tests are not all exactly equal. They are generally comparable as to whether the type was

Fig. 3. Polytene chromosomes from salivary glands of *O. pachycericola.*

significant or not. Clearly crosses between mainland males (*O. longicornis*) and La Paz females (*O. pachycericola*) are the strongest contributors to variation in mating time in these analyses and in Fig. 2.

Species distributions.—Ryckman and Olsen reviewed in some detail distributions of *O. longicornis* in north and central America. They included in their survey specimens from '6 mi. E. La Paz' Baja California, this is in the region we sampled for *O. pachycericola* and probably represents this species. The distribution for *O. longicornis* includes the Sonoran and Mohave desert regions of California and Arizona. Steyskal (1965) gives morphological notes for larval specimens of *O. longicornis* collected in Harlingen, Texas (lower Rio Grande Valley). We have collected specimens of *O. longicornis* from regions around El Paso, Texas and the upper Rio Grande Valley (Starr County) in Texas. This apparently represents the eastern limit for this species in the U.S.

We attempted to discover possible zones of sympatry for these species by collecting or securing specimens from likely areas. We examined larval specimens from San Esteban and Tiburon islands in the Gulf of California, west of Bahia Kino, Sonora, these samples had means of 32.75 (SD = 1.38, n = 8) and 33 (SD =

Fig. 4. Polytene chromosomes from salivary glands of a hybrid formed from *O. pachycericola* and *O. longicornis.*

2.82, n = 2) total spiracular papillae respectively, indicating that these islands are occupied by *O. longicornis.* Larval illustrations of specimens from Los Angeles County, California by Olsen and Ryckman (1963) show the spiracles of their specimens to be within the range for the Sonoran desert samples of *O. longicornis.* Four adult specimens collected by W. T. Starmer from San Telmo, Baja Ca. Norte in 1979 had 36 (SD = 6.73) anepisternal spots, indicating that this region is occupied by *O. longicornis.* Based on this evidence then, we have not found any zones of sympatry for the two *Odontoloxozus* species. Furthermore, we suggest that for northern Mexico and the southern desert regions of the U.S.A. there are still only two known Neriidae, *O. longicornis* and *Onecopsis flavifrons* (Bigot).

Recently one of us (RLM) has collected and examined 23 specimens from columnar cacti (*Lemaireocereus* prob. *pruinosis*) in central Chiapas (6 km SE Tuxtla Gutierrez) averaging 35.48 (SD = 3.53) spots on the left anepisternum.

Table 2. Single factor analysis of variance (ANOVA) results for tests of effects of population, and types of mating on time in copula.

Factors		Statistics (ANOVA df = 1,131)	
Population	Sex	F value	$P <$
Tucson	M	3.221	.075
Tucson	F	9.196	.003
Mazatlan	M	.488	.486
Mazatlan	F	6.645	.011
Kino	M	.220	.640
Kino	F	3.315	.071
La Paz	M	1.022	.314
La Paz	F	58.515	<.001
Type Mating			
Allopatric*		36.861	<.001
Mating#		.832	.363

* La Paz × Non-La Paz (i.e. interspecific).
# Sequence number of mating, e.g. 1 is first mating, 2 is second etc.

Available evidence thus indicates that samples from central Mexico and Central America listed in Ryckman and Olsen (1963) are correctly identified as *O. longicornis*.

Discussion

From this analysis we view the most likely speciation process in *Odontoloxozus* as having been allopatric, there being no evidence of secondary contact. Lack of host plant specificity among populations in this and previous studies (Ryckman and Olsen, 1963) and the transient nature of the cactus substrates suggests that sympatric or stasipatric host plant associated factors were not likely to have been involved in this isolation. In other insect groups, host plant specificity or sedentary habits have been associated with behavioral and chromosomal processes leading to speciation (see White, 1978 for review). In the cape region of Baja California, isolated stands of senita cacti in river flood plains and hecho cacti at higher elevations may have served as substrates for small spatially isolated populations. In these populations chromosomal aberrations could become fixed (see Bush, 1975).

Morphological analysis of the hybrids of these species indicates varying degrees of dominance for certain quantitative characters. Anepisternal spot number for hybrids, pooled for crosses in both directions is slightly higher than, and statistically significant from, *O. pachycericola*. Anterior spiracular papilla number showed less dominance with a mean intermediate between that for *O. pachycericola* and *O. longicornis*. No obvious differences were noted in adult sizes in the species, therefore it is doubtful that nutritional differences are directly responsible for the difference in spiracular morphology. Coefficients of variation for all measurements indicate nearly twice the variation in mesopleural spot number than spiracular papilla number.

Hybrid matings, in addition to producing reproductively inviable offspring, incur extensive costs in terms of mating time investments. Mangan (1979) showed that for *O. longicornis*, male mating fitness can quite adequately be appraised as

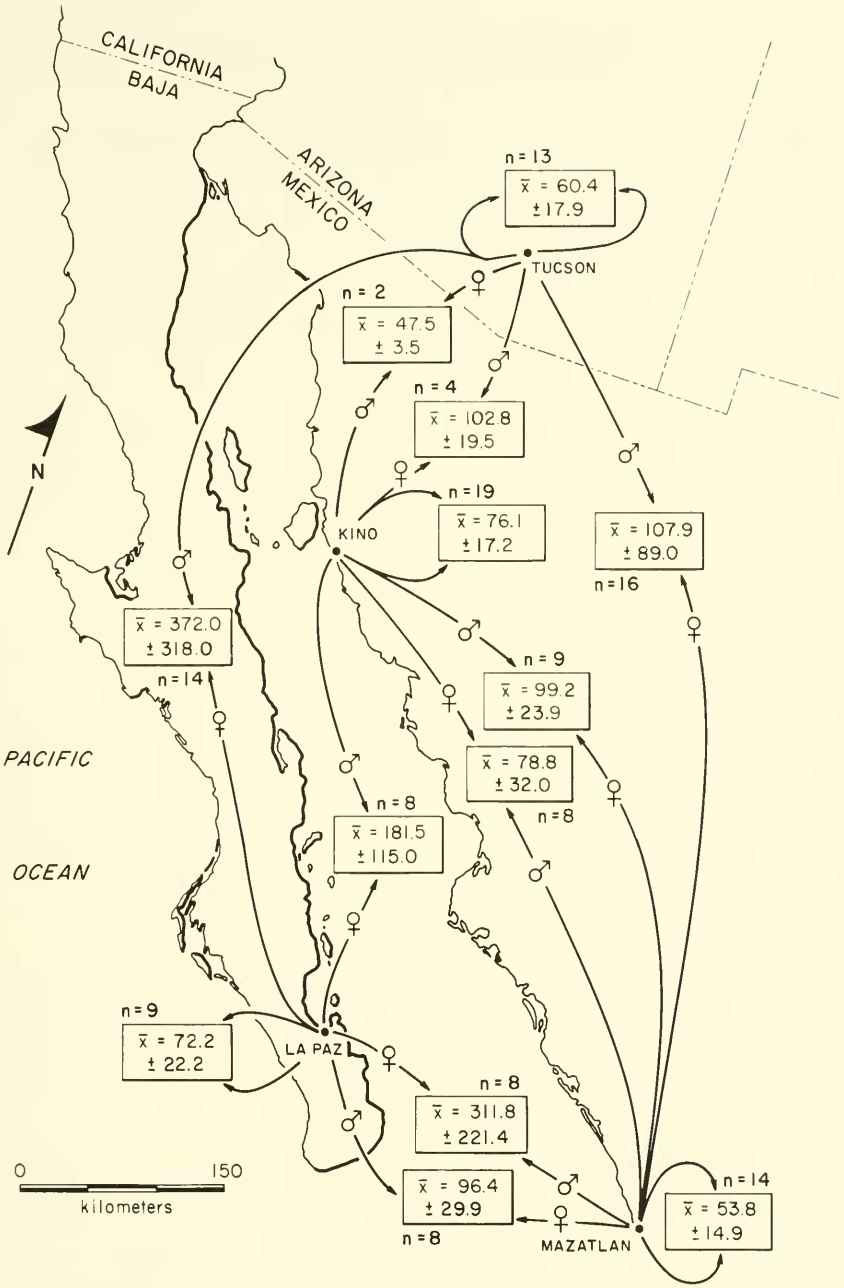

Fig. 5. Mating times (in seconds) from various crosses among Sonoran desert and Baja California populations.

an offspring gain per minute investment ratio. Extended mating time investments by *O. longicornis* males in hybrid matings incur an average of three to eight times the time investment for a normal mating with no gain. We hypothesize that *O. longicornis* males would be under heavy selection pressure to avoid such matings if regions exist where populations of the two species are sympatric. While such selection has been a part of classical speciation theory (Mayr, 1942) this process is not accepted as a part of speciation by all systematists (Futuyma and Mayer, 1980; Paterson, 1978, 1981).

Repeated matings by both gravid and immature females were noted by Mangan (1979) for *O. longicornis*. According to theoretical treatments by Parker (1974) and experimental studies by Prout and Bundgaard (1977) with *Drosophila,* genetically controlled fitness differences in terms of sperm competition in species with multiple mating are significant components of overall reproductive fitness. The lengthening of mating times between individuals from different species or populations separated by long distances (Tucson-Mazatlan) may reflect differences in adaptation of male behavior or physiology to local female reproductive systems. Behavioral and genetic divergence of Tucson and Mazatlan populations suggests the possibility of clinal evolution over the range of *O. longicornis*.

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